INFLUENCE OF SODIUM DODECYL SULPHATE ON THE SEDIMENTATION VELOCITY OF PROTEINS

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1. Introduction

Studies on the sedimentation of a series of proteins in SDS* have revealed species with unexpectedly low sedimentation coefficients (s). In some instances (e.g., [1], low s values obtained in SDS have been interpreted tentatively as corresponding to a polypeptide of low molecular weight. We present here an interpretation of these observed low s values in terms of the hydrodynamic properties of SDS–protein complexes consistent with the model of Reynolds and Tanford [2] for such complexes.

2. Materials and methods

Jack bean urease, bovine liver glutamate dehydrogenase, beef liver catalase, bovine serum albumin, haemoglobin (Type 1), egg white lysozyme, and SDS were obtained from the Sigma Chemical Co., horse heart cytochrome *c* from the Boehringer Corporation and *Escherichia coli* β-galactosidase from the Worthington Biochemical Corporation. Pyruvate carboxylase was prepared by a modification of the method of Scrutton and Fung [3]. Polyacrylamide gel electrophoresis chemicals were obtained from E. Mercke, A.G.

S-carboxymethylation of purivate carboxylase was carried out by the method of Kemp and Rogers [4]. Proteins were electrophoresed on the 'half-crosslinked', 10% polyacrylamide gels described by Weber and Osborn [5]. Gel filtration was carried out using a Sephadex G-150 column (80 × 2.2 cm) equilibrated with 0.1 M sodium phosphate buffer, pH 7.0, containing 0.5% SDS and 0.1% 2-mercaptoethanol.

Sedimentation velocity experiments were conducted in a Beckman Model E ultracentrifuge at 56 100 rpm, with 2.5 mg per ml of protein prepared by incubation in the above SDS-buffer for at least 12 hr at 30°C.

* *Abbreviation*: SDS = Sodium dodecyl sulphate.

3. Results and discussion

Fig. 1A presents the results obtained when a number of proteins were electrophoresed in 0.1% SDS-polyacrylamide gels, and shows that the relative mobility of the proteins through the gel was proportional to the logarithm of their molecular weights. Similarly, fig. 1B shows the elution volume of the same proteins from Sephadex G-150 columns in the presence of 0.5% SDS. These results illustrate that the measured parameters, mobility and elution volume, are functions of polypeptide molecular weight (or chain length) for the group of proteins studied. Under the conditions of the present work, most proteins bind SDS with a high affinity and in approximately constant binding ratio which is 1.4 ± 0.2 g of SDS per g of protein. The critical value for saturation is that the equilibrium SDS-monomer concentration should be at least 8×10^{-4} M (0.023%) [6], which is well below the working concentration of 0.1% or 0.5%. Therefore, at these saturating levels of SDS, the complexes bear a constant charge and electrical force per unit mass, resulting in electrophoretic separation according to hydrodynamic properties (i.e., size), and independently of intrinsic charge.

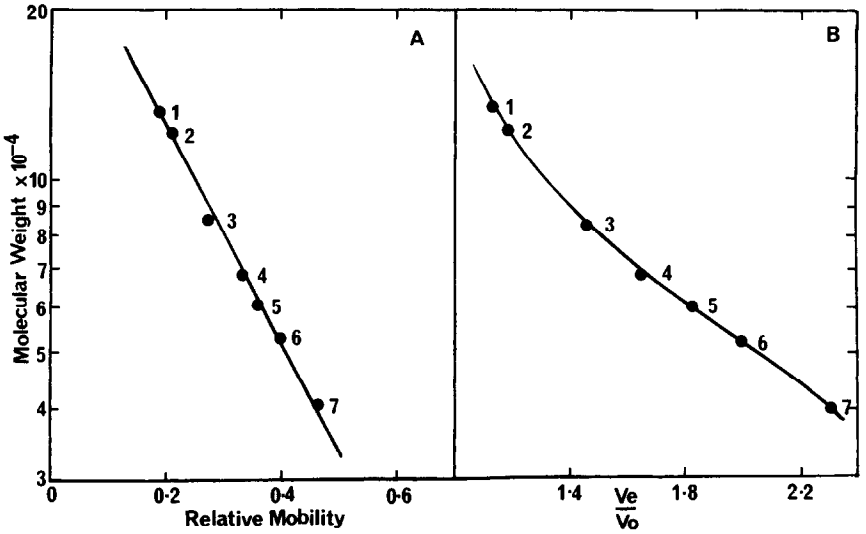

Fig. 1. Semilog plots of molecular weight plotted as a function of: (1) relative mobility of proteins on SDS polyacrylamide gels (A); (2) relative elution volume of various proteins on Sephadex G-150 in SDS (B). The proteins are: 1 – β-galactosidase; 2 – pyruvate carboxylase (chicken liver or sheep kidney); 3 – urease; 4 – bovine serum albumin; 5 – catalase; 6 – glutamate dehydrogenase and 7 – ovalbumin.

Table 1
Comparison of sedimentation coefficients determined for proteins in SDS with the expected sedimentation coefficients

Protein	Molecular weight	Theoretical 'Globular' sedimentation coefficient [a]	Experimental sedimentation coefficient
β-Galactosidase monomer	134 000 [b]	6.32	2.65
Pyruvate carboxylase monomer (sheep kidney)	120 000 [d]	5.88	2.51
Urease monomer	83 000 [b]	4.60	2.50
Bovine serum albumin	68 000 [b]	4.03	2.97
Catalase monomer	60 000 [b]	3.70	2.58
Glutamate dehydrogenase monomer	53 000 [b]	3.41	2.30
Ovalbumin	43 000 [b]	2.96	2.69
Haemoglobin	15 500 [b]	1.42	1.71
Cytochrome *c*	13 400 [c]	1.36	1.80

[a] Determined from eq. (3).
[b] See Weber and Osborn [5].
[c] See ref. [14].
[d] Unpublished observation.

Our studies on the sedimentation velocity of a number of proteins in 0.5% SDS-buffer have revealed unexpectedly low *s* values (table 1). These values can be rationalized by reference to the general equation Mandelkern et al. [7] to describe the sedimentation velocity of macromolecules which are not extremely prolate ellipsoids (eq. (1));

$$\frac{s[\eta]^{\frac{1}{3}}}{M^{\frac{2}{3}}} = \frac{2.5 \times 10^6 (1 - \bar{v}\rho)}{\eta_s N} \qquad (1)$$

where s is the sedimentation coefficients $[\eta]$ the intrinsic viscosity of the solute, M, the molecular weight of the solute, $\bar{v}$, the partial specific volume of the solute, ρ, the solvent density, η_s, the solvent viscosity and N, Avogadro's number. The constant 2.5×10^6, increases very slightly with changes in axial ratio in the case of prolate ellipsoids [8]. Halsall [9] has pointed out that this equation is obeyed by a great many globular proteins, and that to a good approximation it may be simplified to eq. (2):

$$\log s = \log k_1 + \tfrac{2}{3} \log M \quad (2)$$

where k_1 is a constant. By analysis of experimental sedimentation constant data (i.e., $s^0_{20,w}$), an empirical form of eq. (2) can be deduced (eq. (3)).

$$\log s^0 = 3.383 \pm 0.044 \pm \tfrac{2}{3} \log M \quad (3)$$

For comparison, this equation has been used to compute the theoretical sedimentation constants in aqueous solution for the polypeptide chains listed in table 1, assuming them to have globular conformations (Theoretical 'Globular' Sedimentation Coefficients). The large discrepancies between these theoretical values and the observed sedimentation coefficients for SDS–protein complexes are less significant than the fact that the latter values are a much less sensitive function of the polypeptide molecular weight. The magnitude of the s values is, of course, different because the addition of SDS changes the values of η_s, ρ and particularly $\bar{v}$ which reflects the large change in particle volume caused by solvent binding. Furthermore, the different molecular weight dependency is due to the fact that protein–SDS complexes are atypical. Whereas $[\eta]$ for globular proteins is independent of molecular weight, Reynolds and Tanford [2] have shown that $[\eta]$ for protein–SDS complexes obeys the empirical eq. (4):

$$\log [\eta] = \log k_2 + 1.2 \log M \quad (4)$$

where k_2 is a constant and M is the molecular weight of the polypeptide chain as before. Thus, in the case of protein–SDS complexes, eq. (1) is reduced to eq. (5), which becomes eq. (6) by substitution:

$$\log s = \log k_3 + \tfrac{2}{3} \log M - \tfrac{1}{3} \log [\eta] \quad (5)$$

$$\log s = \log k_4 + 0.27 \log M \quad (6)$$

where k_3 and k_4 are constants.

Equations (6) predicts that $\log s$ plotted as a function of $\log M$ for protein–SDS complexes should be a straight line of slope 0.27. Fig. 2 shows such a plot for the data of table 1, and also the similar data obtained by Nelson [10]. The s values of table 1, determined at 21.5°C, and those of Nelson at 25°C have both been corrected to 20°C, the temperature used in the experiment to determine equation (4), by using the approximation given by Schachman [11].

$$S_{20,w} \simeq S_{obs} \frac{(\eta_{t,w})}{(\eta_{20,w})}$$

where $\eta_{t,w}$ and $\eta_{20,w}$ are the viscosity of water at t°C and 20°C respectively. When the data shown in fig. 2 were fitted to a straight line of best fit, shown in the diagram, the slope was found to be 0.30 ± 0.06, which is not significantly different from the value of 0.27 predicted by eq. (6). It is substantially different from the slope of eq. (3) for globular proteins which is shown in fig. 2 for comparison.

The wide scatter of the points in fig. 2 emphasises that we have not attempted a precise analysis of the sedimentation velocity o protein–SDS complexes. Reynolds and Tanford [2] have presented theoretical arguments and experimental evidence which suggest that protein–SDS complexes are long, thin, rod-shaped particles. Interpretation of the sedimentation of such particles is a complex topic which is discussed at length by Creeth and Knight [12]. The situation is complicated by the uncertain effects of the binding of large amounts of the solvent component, SDS, to give a highly electrically charged complex. However, the agreement between predicted and observed slopes demonstrated in fig.2 shows that the molecular weight dependence of the sedimentation coefficients of protein–SDS complexes is at least consistent with their hydrodynamic properties, and hence with the rod-shaped model of Reynolds and Tanford.

Probably a major source of the scatter of points in fig. 2 is the fact that the sedimentation coefficients are not the constants obtained by extrapolation to zero protein concentration, because as Creeth and Knight [12] have pointed out, asymmetric (or expanded) macromolecules exhibit a pronounced decease in sedimentation coefficient with increasing macromolecule concentration. We have confirmed that this is so in the case of a protein–SDS complex by means of the

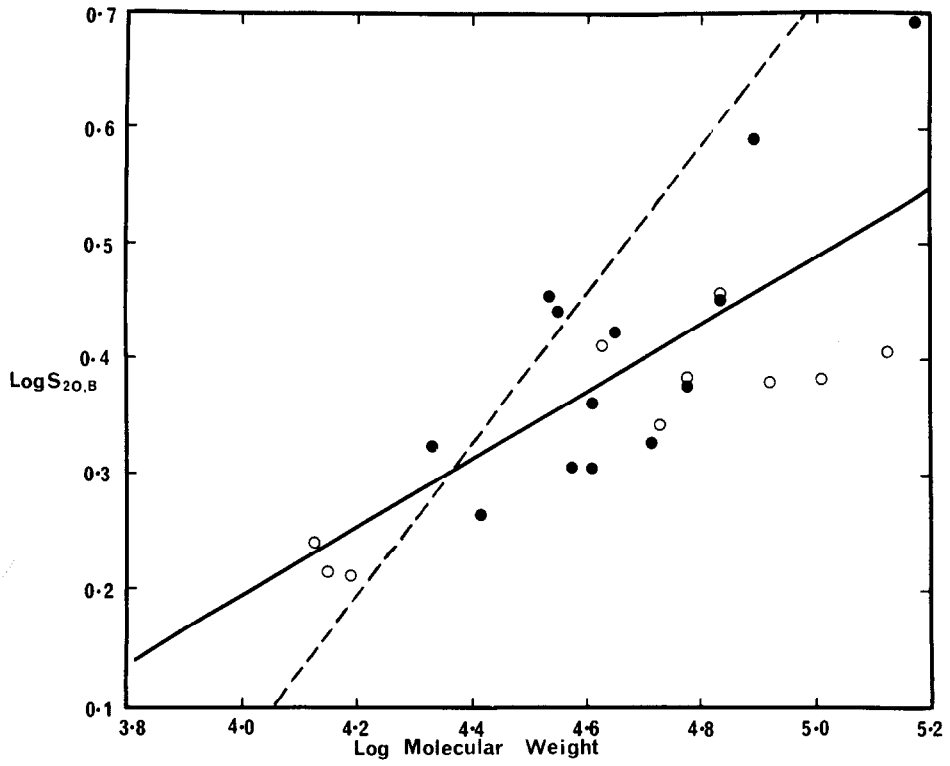

Fig. 2. The relationship between molecular weight and sedimentation coefficient, s for the protein–SDS complexes in table 1 (○) and those of Nelson [10] (●). The data have been corrected for temperature as given in the text. The data when fitted to a straight line (full line) by the method of at least squares gives a value for the slope of 0.30 ± 0.06 which does not differ significantly from the theoretical value of 0.27 ($0.6<P<0.7$ on degrees of freedom). The dashed line represents the equation of Halsall [9] for the sedimentation of globular proteins in aqueous solution.

Table 2
Comparison of sedimentation data for bovine serum albumin in the presence and absence of SDS

	s^0	K_S [a] (ml/g^{-1})	$[\eta]$	$K_S/[\eta]$	Axial [c] ratio
Native protein	4.60	5.4	3.9	1.38	3.2
Protein–SDS complex	2.93	34	54.2 [b]	0.63	45

[a] Determined form the sedimentation data using the equation $1/s = 1/s^0 (+ K_S C)$ given in Creeth and Knight [12].
[b] From Reynolds and Tanford [2].
[c] From the empirical equation given in Creeth and Knight [12].
Log_{10} (axial ratio) = 1.56 (1.7 − $K_S/[\eta]$.

results presented in table 2 for a readily available and well-characterised globular protein, bovine serum albumin. The results are compared with the data of Baldwin [13] for the sedimentation of the native protein, which exhibits less than half the concentration dependence.

Creeth and Knight further state that the ratio of K_S (the coefficient of concentration dependence of the reciprocal sedimentation coefficient, see table 2) the intrinsic viscosity $[\eta]$ for a particle, is indicative of asymmetry, if the value is substantially below that of about 1.6 found for compact spherical macromolecules.

The combination of data presented in table 2 show that the ratio is 1.38 for native bovine serum albumin at 25°C, but 0.63 for the protein–SDS complex at 20°C, and therefore, also suggest that the detergent induces substantial asymmetry in the molecule.

If the empirical equation for axial ratio given by Creeth and Knight, (see table 2), may be applied to protein–SDS complexes, then a very tentative value for the axial ratio of the bovine serum albumin–SDS complex is 45, while that of the native protein is only 3.2.

Acknowledgements

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